ANTIMICROBIAL EFFECTS OF ACACIA NILOTICA AND VITEX DONIANA ON THE THERMOPHILIC CAMPYLOBACTER SPECIES

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ABSTRACT: This study was carried out to investigate the in vitro activity of the extracts of Acacia nilotica and Vitex doniana against Campylobacter jejuni, C. coli, and C. laridis isolated from sheep in Zaria and Kaduna. Water and ethanol crude extracts of Acacia nilotica and Vitex doniana were tested on the thermophilic Campylobacter species. The results obtained show that ethanol extract of Acacia nilotica had minimal inhibition concentration (MIC) of 80 mg/ml, while water extract of the same plant gave an MIC of 250mg/ml. However, ethanol extracts of Vitex doniana had no inhibitory effects on the Campylobacter species tested. A. nilotica and Vitex doniana were used at concentrations ranging from 2 to 200mg/ml of the extracts. Ethanol extract of A. nilotica at concentration of 200mg/ml and 20mg/ml had inhibitory diameters zone of 6mm and 4mm respectively. Water extract of thee same plant at concentrations of 200mg and 20mg had diameters of only 2mm and 1 mm respectively. There was very little or no inhibitions with Vitex doniana water and ethanol extracts. The emergence of Campylobacter strains resistant to most common antibiotics highlights the need to explore new methods for therapeutics against Campylobacter infections. This study has demonstrated that extracts of Acacia nilotica show antibacterial activities against Campylobacter species isolated from sheep in Zaria and Kaduna.

INTRODUCTION

Campylobacter enteritis caused by thermophilic species (C. jejuni, C. coli) is frequently mild to moderate self limiting illness (Pilar et al., 1996). The presence of Campylobacter species among healthy and diseased farm animals has been reported in many countries (Prescott and Brun-Moschi, 1981; Olubunni and Adeniran, 186). Jiwa and Ishengoma (1985), reported the presence of C. jejuni, and C. coli in a number of farm animals including goats at the Sokoke University of Agriculture, Morogoro, Tanzania. In Nigeria, Raji et al., (2000) isolated C. jejuni, C. coli, and C. laridis from sheep in Zaria and Kaduna, Nigeria.

Animals are the commonest reservoir of Campylobacter infections and they may serve as sources of human infection as result of consumption of contaminated meat and meat products (Raji et al., 1997). The use of herbs in the treatment of animal and human diseases has long being used in modern medicine such as cinhona bark for the treatment of malaria and Specacuantia (Ipecac) for the treatment of amoebic dysentery (Zaria et al., 1995). Many plant extracts have been shown to possess antimicrobial agents active against microorganism in vitro. For examples, Little et al., (1988), isolated a naturally occurring 2-methoxy, 1-4 naphthoquinone from Impatinet balamina which is active against several phytopathogenic organisms.

In recent past, some workers in Nigeria have reported results of studies on the effects of Sap and bark of Pycnaathus sangolensis and extracts of Cassia alata inhibited the growth of various types of bacteria including gram negative and gram positive bacteria and also fungi such as Trichopyton species Microsporium species Penicillium species and Aspergillus niger (Babalola, 1988; Sofowora, 1983).

Some extracts of garlic, onion, green pepper and raddish have also been reported to inhibit the growth of
Escherichia coli, Salmonella typhosa, Shigella dysenteriae and Staphylococcus aureus (Sofowora, 1983; Thomas, 1983).

There are about 12,500 of Acacia plant in the tropics and 134 species (represented 170 taxa) native to East Asia (Ross, 1979; Lock, 1991). Acacia nilotica has been reported to be very useful in treating diarrhoea and cough in human (Guinko, 1991). Despite this richness of Acacia species, relatively few appeared to have been investigated. The aim of the present study is to investigate in vitro activity of the extracts of Acacia nilotica and Vitex doniana against C. jejuni, C. coli and C. laridis isolated from sheep in Zaria and at Kaduna abattoir.

**MATERIALS AND METHODS**

a) **Extract Preparation**

Water extract (W.E) and ethanol extract (E.E) of both Acacia nilotica and Vitex doniana were prepared using methods of Tijani-Eniola and Fawusi (1989), and Ibrahim et al., (1983). The extracts of the leaves of Acacia nilotica and the bark of Vitex doniana were used for this study.

b) **Microbiological Assessment**

An aliquot of 0.1ml of 1% of Barium chloride was added to 9.9ml of 1% tetraoxosulphate (VI) to give a McFarland turbidity standard suspension No1. This turbidity approximates bacterial density of about 3X10⁸ organisms per ml. About 0.2ml of the standardized suspension of the bacterial test grown in Nutrient broth were each pipetted onto Muller Hinton Agar plates and spread evenly with aid of glass rods on the agar (Odama et al., 1986).

The paper discs of various concentrations of ethanol and water extracts of Acacia nilotica and Vitex doniana were placed on agar. The concentration range of 2mg, 20mg and 200mg per ml of the extracts was used.

The plates were incubated at 37°C for 24hours and zones of inhibition were then measured to the nearest millimeter using a ruler (Erickson and Sherris, 1971).

The minimum inhibition concentration (MIC) was determined using agar incorporated method as described by Abdulrahman (1986). This was performed by using 0.2ml of the standardized bacterial density of 3X10⁸ organisms per ml. The inoculums were pipette on the Muller Hinton Agar incorporated with the extracts at various concentrations and incubated at 37°C for 24 hours. Following incubation, the growths of Campylobacter organisms on the agar plates with different concentration of the extracts were then observed.

**RESULTS**

Table I and II shows effects of Acacia nilotica and Vitex doniana water and ethanol extracts at concentration of 2mg, 20mg and 200mg per ml respectively on the test Campylobacter organisms used in this study. The results show that Acacia nilotica ethanol extract had profound effect on the isolates. There were no differences observed between the effects of the extracts against the Campylobacter. At concentration of 200mg/ml the diameter of zone of inhibition was 6mm, while for the water extract of the same plant it was 4mm. Vitex doniana ethanol and water extracts had little or no inhibitory effects on the isolates (Table II).

The minimal Inhibition concentration (MIC) for all the isolates was 80mg/ml for the ethanol extract of Acacia nilotica, while water extract of the same plant had an MIC of 250mg/ml at this concentration none of these isolates were able to grow.

The Vitex doniana extracts did not shows inhibitory effect on the isolates even at concentration of 500mg/ml. Bacillus subtilis ATCC strain used as control shows the MIC at 65mg/ml and 125mg/ml for ethanol and water extracts of Acacia nilotica respectively. The Zone of inhibition was also observed for ethanol extract of the same plant and diameter of zone of inhibition was 8mm and 5mm at 200mg/ml and 20mg/ml respectively. The water extract of Acacia nilotica had zones of inhibition of 4mm and 2mm at 200mg/ml and 20mg/ml respectively while the Vitex doniana water extract had no measurable inhibitory effect on the isolates tested. However, the ethanol extract of the same plant had zone of inhibition of 2mm and 1mm. There was no effect on the water extract of Vitex doniana on the isolates.

**DISCUSSION**

The uses of Acacia nilotica have not been supported by any clinical studies and while the successful treatment of venereal diseases, diabetes or use as an aphrodisiac have been documented (Brown, 1977). There are active ingredients present which may be efficacious. Gum, for example, has an emollient activity, resulting in a softening soothing action of the skin or irritated internal surfaces. The astringent activity of tannins causes a contraction of mucous surfaces, coagulates proteins and is useful in stopping bleeding of small wounds and other discharges (Brown, 1977). Acetic acid, alcohol and water extracts of the fruits of Acacia dudgeoni, Acacia nilotica subsp
Antimicrobial effects of *Acacia nilotica* and *Vitex doniana* on the Thermophilic *Campylobacter* species.

Table I. Effect of *Vitex doniana* water and ethanol extracts at various concentrations on *Campylobacter* species isolated from sheep and zone of inhibition.

<table>
<thead>
<tr>
<th>Campylobacter species</th>
<th>Biotypes No</th>
<th>Number of isolates</th>
<th>Ethanol Extract 2mg, 20mg, 200mg per ml and zone of inhibition measure in millimeter</th>
<th>Water Extract 2mg, 20mg, 200mg per ml and zone of inhibition measure in millimeter</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Campylobacter jejuni</em></td>
<td>Biotype I</td>
<td>16</td>
<td>1 0 0</td>
<td>0 0 0</td>
</tr>
<tr>
<td></td>
<td>Biotype II</td>
<td>8</td>
<td>1 0 0</td>
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</tr>
<tr>
<td></td>
<td>Biotype III</td>
<td>4</td>
<td>1 0 0</td>
<td>0 0 0</td>
</tr>
<tr>
<td></td>
<td>Biotype IV</td>
<td>3</td>
<td>1 0 0</td>
<td>0 0 0</td>
</tr>
<tr>
<td><em>Campylobacter coli</em></td>
<td>Biotype I</td>
<td>3</td>
<td>1 0 0</td>
<td>0 0 0</td>
</tr>
<tr>
<td></td>
<td>Biotype II</td>
<td>2</td>
<td>1 0 0</td>
<td>0 0 0</td>
</tr>
<tr>
<td><em>Campylobacter larioidis</em></td>
<td>Biotype I</td>
<td>2</td>
<td>1 0 0</td>
<td>0 0 0</td>
</tr>
<tr>
<td></td>
<td>Biotype II</td>
<td>1</td>
<td>1 0 0</td>
<td>0 0 0</td>
</tr>
<tr>
<td><em>Bacillus subtilis</em></td>
<td>ATTC</td>
<td>1</td>
<td>2 1 0</td>
<td>0 0 0</td>
</tr>
</tbody>
</table>

Table II. Effect of *Acacia nilotica* water and ethanol extracts at various concentrations on *Campylobacter* species isolated from sheep and zone of inhibition.

<table>
<thead>
<tr>
<th>Campylobacter species</th>
<th>Biotypes No</th>
<th>Number of isolates</th>
<th>Ethanol Extract 2mg, 20mg, 200mg per ml and zone of inhibition measure in millimeter</th>
<th>Water Extract 2mg, 20mg, 200mg per ml and zone of inhibition measure in millimeter</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Campylobacter jejuni</em></td>
<td>Biotype I</td>
<td>16</td>
<td>6 4 2</td>
<td>2 1 0</td>
</tr>
<tr>
<td></td>
<td>Biotype II</td>
<td>8</td>
<td>6 4 2</td>
<td>2 1 0</td>
</tr>
<tr>
<td></td>
<td>Biotype III</td>
<td>4</td>
<td>6 4 2</td>
<td>2 1 0</td>
</tr>
<tr>
<td></td>
<td>Biotype IV</td>
<td>3</td>
<td>6 4 2</td>
<td>2 1 0</td>
</tr>
<tr>
<td><em>Campylobacter coli</em></td>
<td>Biotype I</td>
<td>3</td>
<td>6 4 2</td>
<td>2 1 0</td>
</tr>
<tr>
<td></td>
<td>Biotype II</td>
<td>2</td>
<td>6 4 2</td>
<td>2 1 0</td>
</tr>
<tr>
<td><em>Campylobacter larioidis</em></td>
<td>Biotype I</td>
<td>2</td>
<td>6 4 2</td>
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<td></td>
<td>Biotype II</td>
<td>1</td>
<td>6 4 2</td>
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<td><em>Bacillus subtilis</em></td>
<td>ATTC</td>
<td>1</td>
<td>8 6 4</td>
<td>4 2 1</td>
</tr>
</tbody>
</table>
adstringens and subsp nilotica have been shown to have molluscocidal activity. The planting of the later subsp along water ways could prove beneficial in the control of Schistosomiasis (Ayoub, 1982; 1985; Lloos and McCough, 1987). There are numerous medicinal uses of Vitex doniana. The fruits are used to treat anemia and the root is used for gonorrhoea (FAO, 1983). It is also suppose to improve fertility and is used to treat jaundice, and dysentery (Watt and Breyer-Brandwyk, 1982).

Based on the fact that there was no inhibitory effect of ethanol and water extracts of Vitex doniana on the Campylobacter isolates tested at MIC value of 500mg/ml, it is likely that this plant may have no inhibitory or therapeutic effects on the Campylobacter organisms.

The antibiosis of the leaf extracts of Acacia nilotica ethanol and water extracts on Campylobacter species may be related to the antibacterial effect in this plant. This plant has been reported to have saponin, and lipid (Abdulrahman, 1986). The finding that the extracts of this plant showed inhibitory effect at a concentration at 80mg/ml against Campylobacter species showed the potentials of the plant in the therapy of bacterial infection due to Campylobacter organisms.

The susceptibility of Campylobacter organisms to the extract of this plant is very interesting considering the worldwide phenomena of antibiotic resistance of the organism (Coker and Adefosor, 1994). Traditionally the plant material is used as a crude extract and traditional treatment does not aim at using the pure isolate of the extract. Since the objective of this study was to find scientific basis (if any) for the use of this plant material in the treatment, the work did not investigate which of the chemical constituents identified in the plant material could be responsible for the antimicrobial activity.

However, this study demonstrate the in vitro antimicrobial activity of the crude extract of A. nilotica and Vitex doniana against organisms used in the study and it suggests that there is some basis for the use of the extract by the practitioner in the treatment of diarrhoea in human.

In conclusion, this study has demonstrated that the extract of A. nilotica showed in vitro antibacterial activity against Campylobacter species isolated from sheep in Zaria and Kaduna.

Further, detailed investigation is required to identify the active components of this plant. There is also the need to establish standard dosages for herbal preparations and to investigate their toxicity, as toxic manifestation that may result from over dose or from component that may be contained in the plant materials.

REFERENCE

Antimicrobial effects of *Acacia nilotica* and *Vitex doniana* on the Thermophilic *Campylobacter species*.


